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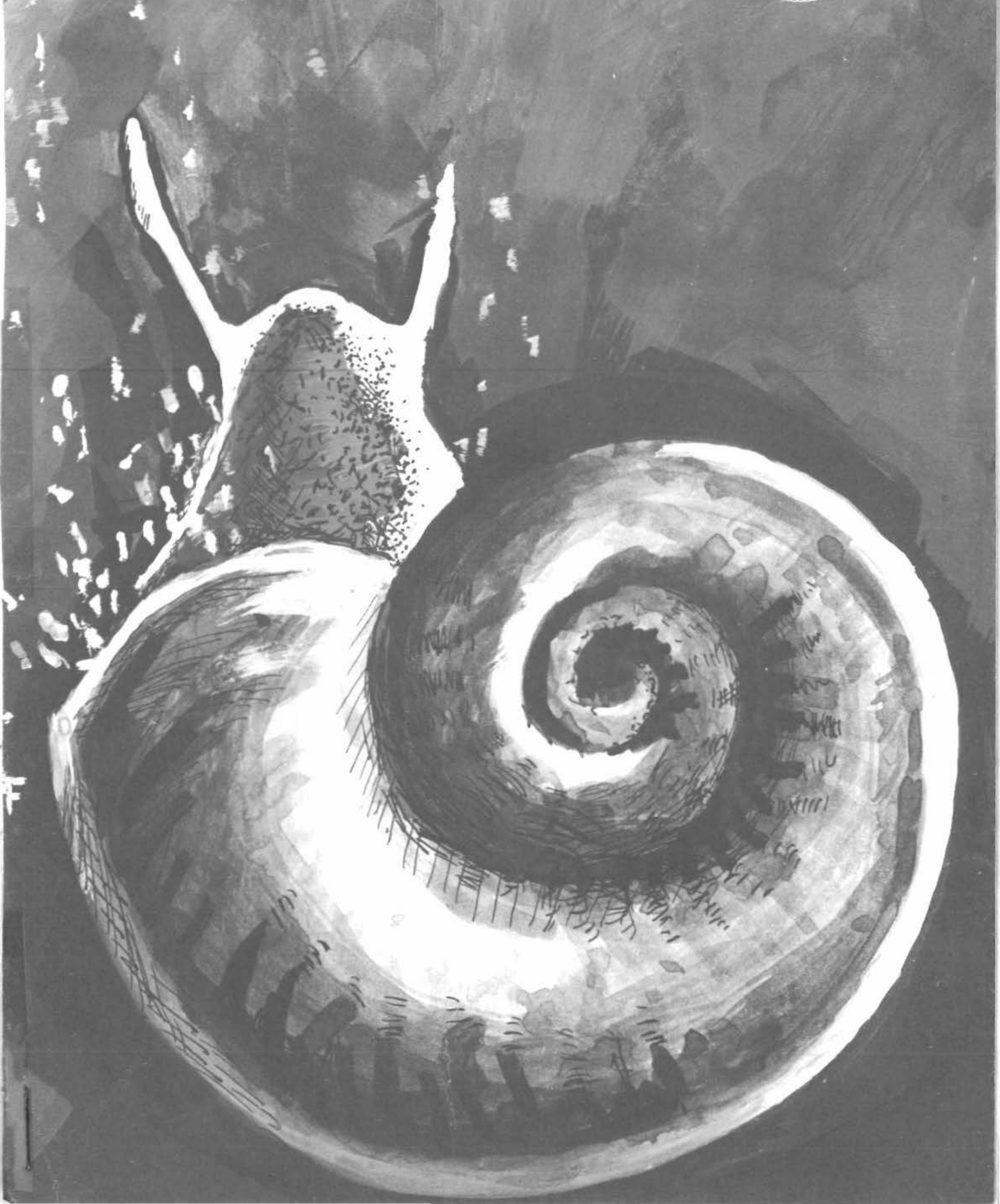

Will Pokeweed Extract Control the Number One Parasitic Disease?

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The Office of Naval Research is supporting the field evaluation of the plant product Endod in the control of the snail-borne disease, schistosomiasis. The objective is to develop practical methods of using Endod for eradicating carrier snails in aquatic environments. Since snails are necessary intermediate hosts, their eradication would break the complex life cycle of the flatworm which causes schistosomiasis. The Navy is supporting this research in order to protect the health and well being of military personnel who are assigned to countries where this disease is endemic.

Schistosomiasis, or bilharziasis, as it is also known, is now considered to be one of the most important parasitic diseases of man – more important than malaria in many countries. In tropical and subtropical regions of Africa, the Far East, the Caribbean Islands, and South America, where the disease prevails, it is spreading rapidly and causing a threat not only to the general well-being of the populace, but also to the economic development of the affected countries. It has been estimated that that about 200 million people in the world are affected by this disease (World Health Organization (W.H.O.), 1965), and the number is increasing rapidly.

Schistosomiasis is a disease caused by infection with parasitic blood flukes transmitted by aquatic snails. Adult male and female pairs of these parasites reside in small blood vessels, and the female has the capacity to lay large numbers of eggs. A single female schistosome is able to lay up to 3,500 eggs per day and has a life span of 3 to 30 years. An infected person may harbor a large number (up to 4,000) of such worms, which – along with the great mass of eggs they lay, and the host's reaction to such foreign material – cause a considerable destruction of the affected tissues.

There are three species of schistosomes affecting man. *Schistosoma mansoni,* widely distributed in Africa, the Caribbean Islands, and South America, causes intestinal schistosomiasis, affecting the liver and mesenteries. *Schistosoma haematobium,* distributed throughout Africa and the Middle East, causes urinary schistosomiasis, affecting bladder, ureters and kidneys. *Schistosoma japonicum*, found in the Philippines, Japan and in the subtropical regions of mainland China, causes a particularly severe form of intestinal schistosomiasis.

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The life cycle involves the excretion of the parasite's eggs in human feces (*S. mansoni* and *S. japonicum*) or in urine (*S. haematobium*). When the mature egg comes in contact with water, it hatches, and the emerging free-swimming *miracidium* searches out and infects an appropriate fresh-water snail. In the snail, the parasite undergoes a complex series of developmental stages, and finally, from each parasitized snail there emerge great numbers of *cercariae*, the form that is infective to man. A single *miracidium* may multiply while in the snail intermediate host to produce many thousands of these larvae, and millions of *cercariae* are continuously released into water by infected snails. There *cercariae* swim about for a day or two in search of their next host – man. On coming in contact with human skin, they penetrate with the help of their proteolytic enzymes, enter the blood stream, pass through the lungs and the liver, and eventually find their way to the small veins in the mesentery or the pelvic region. There, male and female parasites form a semi-permanent copulating pair and develop to maturity. Interestingly, female worms must have male mates in order to mature, though the males can develop independently. A schematic of the life cycle of the parasites is shown in Figure 1.

Owing to the slow development of tissue damage and the chronic nature of schistosomiasis, the extent of the pathology it imposes on infected individuals often is not fully recognized until irreversible liver and tissue destruction has occurred. Though the disease is chiefly a calamitous individual and social burden, shortening the life span but not killing outright, heavy infections can cause mortality, particularly in children. In addition to direct tissue destruction, especially in the liver, schistosomiasis may also reduce resistance to other infections and result in a substantial loss of energy, producing a state of lethargy that is an all too common sight in the Tropics.

Schistosomiasis is a typical example of a man-made disease. Most developing countries depend on agriculture for their economic development. Well-intentioned agricultural projects involving use of irrigation canals have, unfortunately, often resulted in creation of many new breeding habitats for schistosome-transmitting snails. Infected and uninfected people must drink from, bathe, and work in these canals; all too often, human wastes are excreted, dumped, or washed into the same water. This deadly combination is all that is needed for the rapid spread of schistosomiasis. Large scale agricultural schemes are urgently needed, but settlers and laborers must be healthy to realize their economic potential. The debilitating effects of bilharziasis may result in such heavy losses of manpower that economic gains may never be realized.

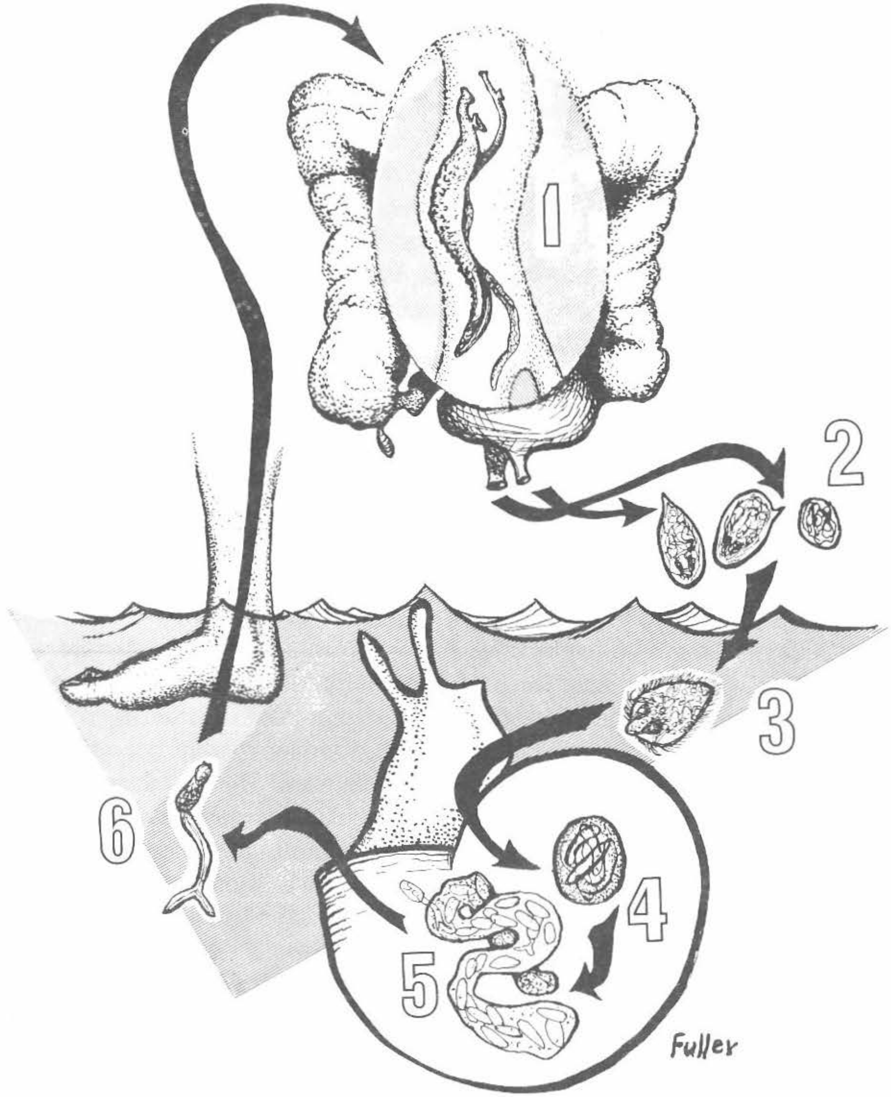

*Figure 1 – This diagram is a simplified life cycle of the schistosomes of man. 1. The adult male and female (*S. mansoni *in the illustration) live in portal vessels (*S. mansoni *and* S. japonicum*) and/or in the vessels of the urinary bladder (*S. haematobium*). 2. The eggs work their way through the bowel (*S. japonicum*-right, and* S. mansoni*-middle) or the bladder wall (*S. haematobium*-left) and are thereby excreted. 3. On reaching water, the miracidia are released and penetrate the soft parts of the snail. 4. Within the snail, mother sporocysts are formed. 5. These in turn produce the more elongate daughter sporocysts which release the infective cercaria. 6. The cercaria on bursting from the snail, swim about and invade the skin of the human host, migrate through cutaneous vessels, thus to the heart and blood vessels where the cycle is repeated as the young flukes mature in the vessels of the portal system or bladder depending on the schistosome involve.*

Conservative estimates of the annual economic loss due to schistosomiasis, based on the reduction of work-output by infected individuals and the general health damage it causes are as follows: in Egypt, about 80 million Egyptian pounds; in Iraq, 6 to 10 million Iraq dinars; in Japan, 18 million U.S. dollars; and in the Philippines, 13 million pesos (Farooq, 1964; Ansari and Junker, 1967).

There are many examples that illustrate the growing economic and public health importance of schistosomiasis. In the Aswan Province of Egypt, *S. haematobium* increased from four to forty fold within three years after introduction of perennial irrigation (Blair, 1964), and the prevalence has now risen to over eighty percent of the population (Malek, 1972). The "ecological nightmare" created by the Aswan Dam has been recently described by Heyneman (1971). About the same scale of increase in infection with schistosomiasis has also been shown for the Gerzera area in the Sudan. Another area where the disease is on the increase is the newly developing Awash Valley of Ethiopia (Lemma, 1969). When prevalence of bilharziasis began to reach alarming proportions in the Umshandige Irrigation Scheme in Rhodesia, the project had to be abandoned in 1949, after ten years of construction costing about 10 million U.S. dollars (W.H.O., 1965; Weir, 1969).

There is now no single effective way to control schistosomiasis. There is no suitable drug for mass treatment, as those available are of high toxicity and require sustained treatment and careful supervision of the patients. In any case, those treated would readily become reinfected as long as the source of infection remained. Health education and sanitary improvements would help immensely, but considering the low levels of education and standard of living of the 400-500 million people exposed to potential infection with this disease, along with the basic human dependence on water and demands made by the need for rapid agricultural development, control of schistosomiasis through education and sanitary improvements alone is unrealistic. Perhaps the single most promising approach for control of schistosomiasis is to interrupt the life cycle of the parasite by eliminating the vector snail.

Among the molluscicides developed over the past 50 years for the control of schistosomiasis and other snail-borne diseases, the oldest and most widely used are copper sulfate and sodium pentachlorophenate. New and much more effective molluscicides, Bayluscide$^{(R)}$ and Frescon$^{(R)}$, have been introduced to the market recently.

Practically speaking, any one of these molluscicides could be used successfully to control snails, and all have been so used. The key to successful use is repeated and carefully monitored use, made possible only by hard work and sufficient funds, necessitating the continuous purchase of large quantities of imported molluscicides. The hard currency needed for this is a critical matter for hard-pressed national budgets of the affected developing countries. Hence, a molluscicide that could be locally produced and used as needed would be ideal. To this end, during the past eight years we have been studying and developing the potentials of a promising plant product molluscicide, Endod, for possible use in the control of schistosomiasis on a sustained "self-help" basis.

Endod is the Ethiopian name for the soapberry plant, *Phytolacca dodecandra,* berries of which are widely used as soap (Figure 2). In 1964, while making an ecological study of the distribution of schistosome-transmitting snails in a small stream in northern Ethiopia, the author observed large numbers of dead snails in areas immediately below the spots where people washed clothes with Endod. The areas up stream and further down stream from such spots had abundant live snails. This led to the discovery of the molluscicidal property of Endod. Since then, there have been numerous studies done on this subject (Lemma, 1965a; 1970a,b; 1971; Lemma and Duncan, 1970; Yohannes, 1970; King, *et al.* 1968).

Sun-dried, crude, ground Endod berries suspended in aqueous solution kill schistosome-transmitting snails at concentrations of 15-30 pounds per million within 24 hours. This molluscicidal potency remains stable over a wide range of pH (5 to 9) values, in the presence of various concentrations of organic and inorganic matter, and after irradiation with ultraviolet light. Its toxicity to mammals and plants is very low. The LD_{50} (the concentration that kills 50% of test animals) is 2.6 g/kg body weight for mice, 6.5 g/kg body weight for sheep and 2.5 g/kg body weight for monkeys. Birds normally feed on the berries in the forest. Continuous application of highly concentrated solutions also appeared not to affect a varicty of plants of economic importance (Lemma, 1970a; Yohannes, 1970).

Encouraged by the preliminary results, we undertook a relatively largescale field trial in Adwa, an agricultural community of 17,000 in northern Ethiopia. From previous investigations (Lemma, 1965b; Buck *et al,* 1965), it was known that approximately 65% of the population were infected with *Schistosoma mansoni.* The sources of infection are two relatively small and isolated streams. They originate from springs and run across the middle of the town and they are extensively utilized by both adults and children. Great numbers of *Biomphalaria pfeifferi,* the Ethiopian vector snail, breed in these rivers and up to 60% of them were infected. A program was developed to control those snail populations by systematic application of Endod over a five-year period. Beginnining in 1969, we checked the effectiveness of these efforts by observing the incidence of disease in the human population, particularly among children one to five years old. To establish baseline data before applying the molluscicide, we determined the prevalence rate of infection in 10% of randomly picked inhabitants. We used aerial photographs to ensure

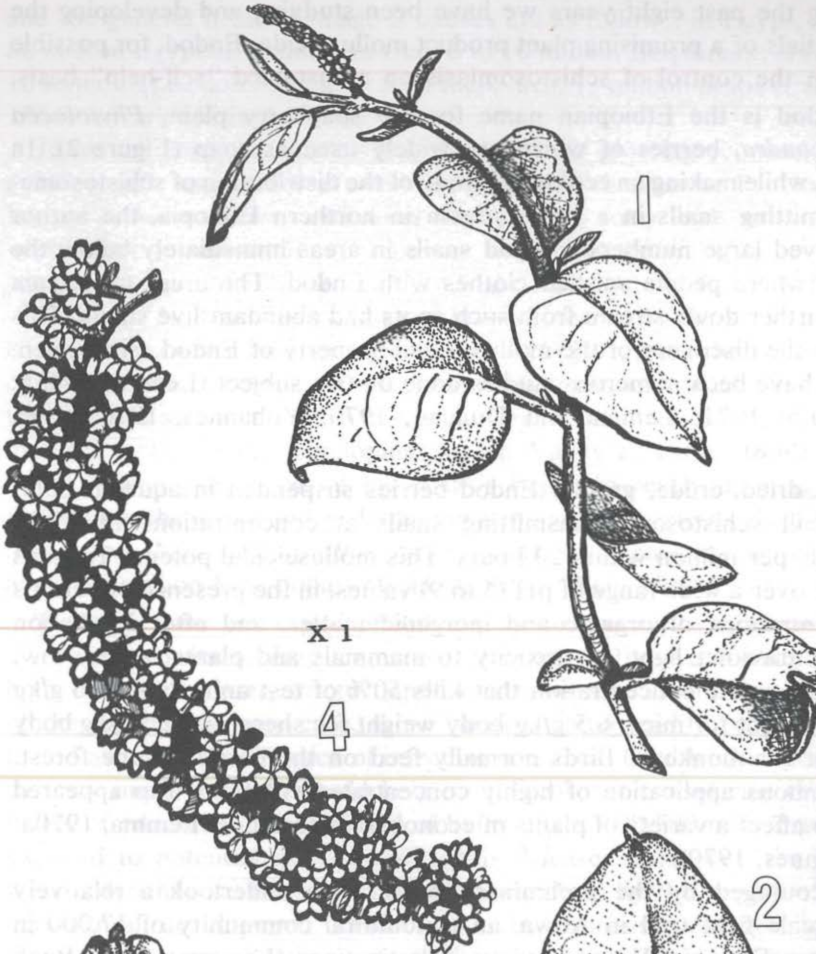

Figure 2 – Phytolacca dodecandra *(Endod plant). 1. Distal portion of a branch showing the leaves and immature fruits. 2. Dorsal surface of an individual leaf. 3. Dried fruit of individual flowers (enlarged ×3). 4. Infructescence natural size.*

every house in Adwa was numbered and located. Houses were selected randomly; the families were identified, and stool specimens were collected and examined for eggs. About 70% of the total population had *Schistosoma mansoni,* and 48% of the children between the ages of 1 and 5 were infected.

Application of the molluscicide involved very simple procedures, so that the local residents could continue the program. The dried berries were ground and suspended in water. Proper concentrations were poured from buckets along the shores or made to flow at a constant rate from barrels placed above the river. Using a concentration of about 100 pounds per million (ppm) for a 4 to 6 hour exposure time, in a few days almost all the snails were killed and washed downstream.

An interim evaluation of the Adwa project made two-and-half years after its start, revealed the following: (1) Endod is effective in controlling the snail population; (2) continuous application of this molluscicide did not have any apparent effect on the flora and fauna of the rivers; (3) the control program resulted in the reduction of the incidence of schistosomiasis from 48% to 15% among the 1- to 5-year old children in Adwa, while the incidence of disease among untreated children in the nearby control village of Intitcho increased by 10%; and (4) rates of infection with other parasites (intestinal worms) in both Adwa and the control village of Intitcho remained the same, thus showing the specific control of schistosomiasis (Flemings and Goll, 1971).

During 1970-72 at the Stanford Research Institute in California, we undertook the following interdisciplinary chemical and biological studies:

1. to determine the structures and functions of the active principle in the Endod berry.
2. to develop an extraction procedure to concentrate the active components,
3. to determine fish and mammalian toxicity of the purified material, and
4. to develop a chemical assay for these components when in aqueous solutions.

Our studies to date indicate that one of the main active components in the berries may be an oleanolic acid with three glucose units attached to it. The exact location and relationship of these sugars to the oleanolic acid is now being determined (Parkhurst, *et al,* in preparation).

A simple and effective procedure to extract and concentrate the molluscicide material from the berries has been developed. This involves butanol extraction of an aqueous solution of the berries, followed by an ether wash to remove inactive, fat-soluble impurities. The butanol extract is a light tan powder, about 20% of the original berry weight.

Its potency (LC_{90}* for 24 hours) against different species of snails is 2-3 ppm as opposed to 15 to 30 ppm of raw berries (a seven to tenfold increase in potency). We have made a considerable effort to simplify the extraction procedure to make it amenable to inexpensive utilization in the field (Lemma *et al,* 1971).

Since Endod is a natural plant product, the berries can be grown and harvested locally and, with proper genetic studies, strains with increased molluscicidal potency can be selected and cultivated for largescale use. Purification of the berries is simple. The major solvent necessary for the extraction, butyl-alcohol, can readily be produced locally by microbial fermentation of molasses (an industrial waste-product in many developing countries).

Unlike other molluscicides, Endod has an important added advantage in that it is a useful detergent. It has no deleterious effect on colour or fiber of delicate fabrics. It is also locally believed that clothes and human hair washed with Endod become free of lice and ticks, and there is, in fact, some experimental evidence to support this. Thus, Endod might be developed and processed for wide-scale use as soap.

Endod will also kill the larval stages of the schistosome parasite at very low concentrations; 1 to 2 ppm of the butanol extract is sufficient to kill *schistosome* miracidia and cercaria. Human waste and polluted water can be treated with Endod and rendered free from these larval stages of the parasite, thus interrupting its life cycle at yet another important stage. Also, a 5% concentration of the extract with an ointment base may be topically applied to the skin to prevent cercarial penetration.

Another advantage of Endod is that it is highly toxic to the aquatic and land leeches that are important pests of domestic animals and man in the tropics.

Unlike some of the other molluscicides and the chlorinated hydrocarbon pesticides, Endod has the advantage of being rapidly biodegraded. This has been shown to be primarily due to bacterial degradation of the sugar components of the active principle. In a comparative test, 0.2 ppm of Bayluscide, another molluscicide, remained active for more than three months.

In view of the encouraging results and promising potential of Endod, additional studies should now be undertaken. One of the most important would be to determine the short- and long-range ecological effects of continuous application of the molluscicide on the flora and fauna of the treated bodies of water. The development of improved strains of Endod by controlled genetic selection should be undertaken, and agronomic studies to determine the environmental factors that affect the growth and production of the plant should be encouraged. Adequate methodology should be developed for preparation of the purified product at the village level, along with a program to encourage its use as soap, and as a chemoprophylactic for protection against cercaria. The possibilities of developing synthetic analogs of the glucoside of oleanolic acid for a more effective biodegradable molluscicide not toxic to fish should also be explored.

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*Lethal concentration of Endod necessary to kill 90% of test organisms.

The organic chemistry, biochemistry, and pharmacology of the triterpenoid spaonins of Endod berries should be fully studied. For example, the roots of the Endod plant, which contain saponins, are widely used for medicinal purposes in Ethiopia and elsewhere, and are believed to be highly toxic to mammals, including man. But these roots do not have any effect against snails, even in a concentration as high as 1000 ppm, whereas the berries, which are not toxic to mammals, kill snails at 15-30 ppm. Furthermore, saponins from the berries of a closely related species, *Phytolacca america* (*P. decandra*), have no activity against snails. The relationships and specificity of these saponins should be investigated.

Studies to determine the basis for the specificity of the saponins from Endod and its mode of action against snails, may provide further valuable information that may in turn enable us to improve the molluscicidal potency of Endod and to reduce its toxicity to non-target organisms. This may also guide future searches for alternative sources of such a product.

The ultimate goal of our studies is to develop an integrated program of schistosomiasis control on a community self-help basis. Schistosomiasis involves interaction and activities of humans, their communities, and their developmental programs. Any effective control will depend on cooperative participation by the affected communities. A multiple approach to the problem must be sought. Development of a health education program, based on local cultural and behaviorial aspects, and water-contact and sanitation habits of the people, is essential. Mass treatment of infected individuals, when an appropriate drug becomes available, must also be on the program. The elimination of vector snails by use of chemicals such as Endod, perhaps tied to carefully planned biocontrol systems to disrupt the snail host and/or the schistosome parasite, is another important aspect.

Community involvement through health education, active participation by the people involved, and the systematic application of locally grown and processed Endod should become routine. These activities by individuals, families, and villages, when developed into a long-range control of the disease, become truly a self-help form of health control by the people themselves. The work in Adwa has already pointed the way, a path we shall actively pursue.

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